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ABSTRACTS OF PAPERS

Tenth Annual Albert L. Tester Memorial Symposium 11–12 April 1985[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as on the quality of the public presentation. Judges include several members of the faculty as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year George Barlow of the University of California, Berkeley, participated in the symposium.

Benthic Community Zonation Around Hydrothermal Vents at a Submarine Volcano[2]

ANNE MICHELLE ARQUIT[3]

More than 10,000 bottom photographs taken from Axial Caldera of the Juan de Fuca Rift (44°55′ N, 130°01′ W) were examined to test the hypothesis that benthic biota common to the deep-sea environment are distributed in a zonal pattern near high- and low-temperature hydrothermal vents. Previous research has shown that "exotic" vent communities are distributed zonally, with pogonophorans (gutless "tube worms") nearest high-temperature vents and clams (*Calyptogena pacifica* at the Axial Caldera location) or other bivalves further away. The question to be answered is this: To what distance from a discrete vent are biological processes significantly impacted by environmental changes associated with hydrothermal circulation?

Axial Caldera is an ideal site for a faunal density study, because its 3.5-km-wide, very flat floor minimizes topographic interference, and allows high-quality photographic coverage. Thirty-five-millimeter color slides from a camera sled towed about 5 m above the ocean floor (four frames/min) and additional slides taken by the ALVIN submersible bow cameras (eight frames/min) constitute the main data base. Each photo covers a section of the ocean floor about 3 m by 5 m; the total area studied is about 24 km^2.

Density distribution was determined by reference to several index populations selected

[1] Manuscript accepted July 1985.
[2] This research is supported by a NOAA grant, NA80AA-H-000075.
[3] Department of Oceanography.

for their ease of identification from slides: sponge, shrimp, crinoid, sea urchin, sea anemone, holothurian, starfish, brittle star, and rattail fish. A benthic sponge, *Bathydorus* sp., was the main index used, because this organism is ubiquitous yet its density varies from a few individuals to as many as 800 per slide. The population of index organisms was mapped and analyzed spatially with respect to the location of known hydrothermal vents, both low and high temperature.

Bathydorus sp. sponges are absent from the immediate vicinity of low-temperature vents (that is, vents releasing fluid at temperatures 2–3°C above the ambient bottom temperature of about 4°C), as well as from areas covered with iron oxide and/or silica chimneys and blankets. A progressive increase in the abundance of sponges is seen with distance from the hydrothermal areas; the greatest density of these organisms is 200 to 400 m from the vents. This zonation may be caused by the unfavorable chemical effect of nontronite/iron oxide material on normal benthic organisms near the vent, coupled with an *enhanced* growth environment a short distance from the vents, resulting from increased nutrient levels supplied by advection/mixing of hydrothermal fluids with ambient bottom water.

Palynology of Selected Horizons from the Ewa Coastal Plain, Oahu, Hawaii

THECLA M. BENNETT[4]

A 1070-ft-long core, comprised of both marine and terrestrial sedimentary deposits, from the Ewa Coastal Plain, Oahu, was sampled at 29 horizons for pollen grain and fern spore content. Preservation of palynomorphs was much better below 620 ft than in the upper portion of the core. Palynomorphs were recovered from 16 of 21 samples below this level and from 3 of 8 samples above it.

Thirty-nine palynomorph types were recovered; 10 of the types were found in a minimum of 7 of the 19 horizons which contained palynomorphs. These were *Osmanthus*, *Xylosma*, *Lycopodium cernuum*, and an unidentified fern, all found in 7 horizons, between 622 and 920 ft depth; *Cibotium*, 7 horizons, 623–920 ft; *Broussaisia* and *Ilex*, 8 horizons, 622–920 ft; "cheno-arms," 9 horizons, 166–968 ft; a second unidentified fern type, 10 horizons, 622–920 ft; a third unidentified fern type, 11 horizons, 622–937 ft. The association of these common types of palynomorphs suggests that a basic vegetation type (*Metrosideros* forest) similar to that on parts of Oahu today existed during the Pleistocene.

Phylogenetic Relationships Among the Chaetodontidae (Pisces: Perciformes): A Cladistic Interpretation of Osteology[5]

STANLEY D. BLUM[6]

W. E. Burgess (1978. Butterflyfishes of the world. T. F. H. Publications, Neptune City, New Jersey. 832 pp.) recognized 114 valid species within the Chaetodontidae. He classified the family into 10 genera and further divided one of these, *Chaetodon*, into 13 subgenera. I have completed an osteological survey which encompasses almost every species group in the family, including at least one representative from each of these taxa, as

[4] Department of Botany.

[5] This research was supported by a Smithsonian Predoctoral Fellowship and a grant from the Coral Reef Foundation.

[6] Department of Zoology.

well as a number of fishes from outside the family.

Specimens were prepared for examination using a standard procedure, called counterstaining, that turns cartilage blue and bones red and leaves most of the soft tissues clear. Dissections were performed to enable the examination of all gross osteological morphology except the internal features of the cranium.

Hypotheses of character homology were made as complete as possible by specifying character transition series. The transition series were then converted into numerical form by additive binary coding. The information from all characters was combined, without weighting, into a single data set. A computer program was employed to find the phylogenetic hypothesis that requires the smallest amount of convergent and atavistic character evolution.

The resultant phylogeny is shown to be congruent with Burgess's classification at the generic level but incongruous at the subgeneric level. Specifically, the subgenus *Chaetodon* is shown to be paraphyletic. If the classification of the family is to reflect natural groupings and cladistic principles, the taxonomy at this level will have to change.

Analysis of 1-Canavanine Resistant HeLa Cells[7]

ROBERTA LYNN BRASHEAR[8]

Clonal mutant HeLa cell lines, resistant to the deleterious arginine analog 1-canavanine, were obtained by the ultraviolet mutagenesis and analog selection method described by Yim and Stuart (Biochem. Genet. *21*:443, 1983). Five stable clonal lines were isolated and tested for resistance to 1-canavanine at 35°C and 39°C. Two cell lines demonstrated resistance to the analog at a concentration of 6×10^{-3} *M* at both temperatures. A third line was resistant to concentrations up to 3.5×10^{-3} *M* at both temperatures. A fourth line showed partial resistance under the same conditions. A fifth line showed temperature sensitivity with partial resistance to the analog at a concentration of 2.5×10^{-3} *M* when grown at 39°C but no resistance at 35°C. The parental HeLa cell line from which these mutants were derived does not survive at either temperature in the presence of the analog at a concentration of 2.5×10^{-3} *M*. Changes in cell morphology also were demonstrated. Some of these mutant cell lines also demonstrate defective levels of arginine transport and uptake. These analyses may be useful in understanding the genetic and biochemical pathways associated with heritable transport disease.

Paradigm Clash in the Systematic Triumvirate: Classifications of the Caesionidae (Pisces: Lutjanoidea)

KENT CARPENTER[9]

Three schools of systematics currently vie for acceptance as the reigning paradigm in biological taxonomy. Based largely on philosophical grounds, each of these schools, the evolutionary, phenetic, and phylogenetic, claims to produce superior classifications. Although Linnean classifications produced from them can differ widely, there has apparently been no attempt to evaluate all three

[7] This research was supported in part by funds from the University of Hawaii.

[8] Department of Genetics.

[9] Department of Zoology.

simultaneously using general, objective optimality criteria. The purpose of this study is to compare classifications produced by the different approaches using numerical criteria based on predictive value and information content, which are goals common to all three schools.

The taxonomic units used in this study are the species of the family Caesionidae (Pisces: Lutjanoidea). This family contains 20 species in four generally accepted genera. The phylogenetics and higher taxonomy of the group have previously been studied using classical taxonomic procedures. Thirty-six meristic, morphometric, and osteological characters were surveyed and coded into 95 binary character states. Linnaean type classifications were produced based on the tenets of the three schools of taxonomy. The hierarchical patterns and taxonomic groups inherent in the most common interpretation of these classifications were evaluated using two numerical optimality criteria. These criteria included a recently developed predictive value index based on maximizing homogeneity within taxa and heterogeneity among taxa and an index based on information theory.

The phenetic and evolutionary systematic classifications had similar predictive values and information content, while the phylogenetic classification scored consistently lower under these criteria. These differences were significant ($P < 0.01$) using a nonparametric pairwise comparison statistic. Therefore, the phylogenetic Linnaean classification would be rejected while the choice between either the phenetic or the evolutionary classification must be based on a philosophical decision.

The Effects of Fish Grazing on Juvenile Corals

RACHEL FITZHARDINGE[10]

Fishes belonging to the families Scaridae, Acanthuridae, and Siganiidae are important grazing organisms on coral reefs. The impact of their grazing has been described as being both favorable as well as detrimental to the growth and survival of juvenile corals. This discrepancy in the literature may result from researchers investigating grazing on corals that differ in size and age. Young corals less than 2 mm in diameter may not be readily identified as corals by herbivorous fish and therefore may be taken indiscriminately with algae and other food items. Once corals reach 3–4 mm in diameter they may become large enough for herbivorous fish to recognize and avoid ingesting them. At this size, coral growth rates are slow compared to the more rapidly growing algae and other sessile invertebrates that compete for space with the corals. Grazing may then begin to favor coral growth and survival by removing species which compete for space with corals or which overtop and shade them.

The relationship between the intensity of fish grazing and successful coral recruitment has been investigated in Kaneohe Bay by using concrete blocks as experimental substrata. Eight blocks were placed at seven shallow-water sites on three patch reefs during the spring of 1983 and 1984. The blocks were examined after 3 and 6 months, and the 1983 blocks were also examined after 15 months. The number and species of coral recruiting to each surface of the block were recorded. Algal cover on top of the blocks, which is the surface most exposed to grazing, was recorded using a point quadrat method. Three types of algal cover were recognized. The first was "algal turf" consisting of green, brown, or blue-green algae less than 1 mm in height. Coralline algae and other encrusting red algae com-

[10] Department of Zoology.

prised the second group. The third group. "macroalgae," comprised all algae over 1 mm in height. Herbivorous fish abundances were estimated at each site by counting the number of fish seen within 4 m of a transect 30 m long.

Pocillopora damicornis was the only species of coral found on the blocks after 3 months in 1983. In 1984 *Cyphastrea ocellina* was also present on the blocks after 3 months. The recruitment of *Pocillopora damicornis* to the blocks after 3 months lends some support to the hypothesis that fish grazing may cause mortality to newly settled corals 1–2 mm in diameter, since the highest number of *P. damicornis* recruits of this size were found in both 1983 and 1984 at sites with low abundances of grazing fish. Recruitment rates at some sites did differ greatly between years, however, perhaps reflecting differences in the abundance of planulae. The most abundant grazers were parrotfish (*Scarus* sp.) and surgeonfish (*Acanthurus triostegus* and *Zebrasoma flavescens*). These fish were most common around the margins of reefs except where they were excluded by the territorial damselfish *Stegastes fasciolatus*. They were rare or absent at the reef flat sites. Blocks subject to frequent fish grazing also had a high cover of coralline algae whereas those that were little grazed by fish and invertebrates had a high cover of macroalgae. Three additional species—*Porites compressa*, *Montipora verrucosa*, and the ahermatypic coral, ? *Culicia* sp.—were recorded from some of the blocks after 6 and 15 months. At one site, a decline in the number of *P. damicornis* colonies over 12 months may be attributable to algal smothering. At most sites where grazing fish were uncommon, however, new recruitment of *P. damicornis* offset any mortality due to overgrowth by competitors. Further manipulative experiments are necessary to clarify how fish grazing affects juvenile corals.

The Effects of Aggregations on Water Loss from the Limpet *Collisella digitalis*

W. Brad Gallien[11]

Collisella digitalis forms conspecific aggregations in the high intertidal splash zone. This research tests the hypothesis that aggregations reduce water loss rates by comparing solute concentrations of extracorporeal water (ECW) of clumped and isolated limpets. The research was conducted for Biology 100, a course offered by the University of California, Berkeley, and the Bodega Marine Laboratory.

Both isolated and clumped limpets started the exposure period with an ECW solute concentration of 1117 mOsm/kg (16% above seawater). After a given exposure period, limpets within conspecific aggregations tended to have lower ECW solute concentrations than nearby, isolated individuals. This finding indicates that over the exposure period clumped limpets had lost less fresh water by evaporation than isolates. The concentration differences ranged from 532 to −20 mOsm/kg for the 21 comparisons between clumped and nearby isolated limpets ($n = 163$). Subtracting the starting concentration (1117 mOsm/kg) from the final solute concentrations, the change in solute concentration over the exposure period can be estimated. For limpets with equal exposure times, these concentration changes can be used to estimate the relative rate of water loss between clumped and isolated limpets: limpets within aggregations lost water at one-third the rate of isolates.

[11] Department of Zoology.

These differences in water loss rates are most likely attributable to reduced wind velocities within the aggregation. Limpets on the perimeter of an aggregation are exposed directly to the wind, slowing it due to drag. Wind velocities within the aggregation would thereby be reduced, and consequently rates of evaporation would also be reduced. The finding that perimeter animals had higher ECW solute concentrations than limpets within the aggregation supports this hypothesis, as do preliminary biophysical analyses.

In addition, isolated limpets tended to orient head down on vertical surfaces, whereas clumped limpets showed no such trend. It has been postulated that the head-down orientation minimizes the chance of ctenidial damage as ECW is lost due to evaporation. Consequently, the trend to orient head down supports the hypothesis that isolated limpets are subjected to more severe desiccation regimes than clumped limpets.

In conclusion, the formation of aggregations is a behavioral adaptation which, by limiting water loss, reduces the rigors imposed by the limpets' semiterrestrial environment.

The Larvae of Ommastrephid Squids from Hawaiian Waters[1,2]

ROBERT F. HARMAN[1,3]

Squids belonging to the family Ommastrephidae are among the largest and most numerous cephalopods in the open ocean. They are fast-swimming animals feeding extensively on fish, crustaceans, and cephalopods. These high-level predators are themselves important prey items for other predators such as tunas, birds, and toothed whales. Additionally, members of this family are targets of commercial squid fisheries in several areas of the world, including Hawaii. Despite their importance, knowledge of the biology and ecology of these oceanic squids is very limited. Large information gaps result from our inability to sample effectively the fast-swimming juveniles and adults. The larvae of these squids, however, can be adequately sampled with standard plankton gear.

This study focuses on means of identifying the larvae of the three ommastrephid species in Hawaiian waters (*Hyaloteuthis pelagica*, *Nototodarus hawaiiensis*, and *Sthenoteuthis oualaniensis*). The larvae of ommastrephids, termed rhynchoteuthion larvae, are distinguished from other squid larvae by the fusion of the two tentacles into a proboscis-like "roussel." The tentacles are initially fused along their entire length, ending in a disk which bears eight suckers. The roussel divides as the squid grows, and the suckers of the terminal disk become part of the tentacular club.

The relative size differences of the two lateral suckers on the terminal disk to the six medial suckers provide an effective means for identifying each species. Other valuable characters are the time of appearance and number of ocular and visceral photophores, the number of knobs on the chitinous sucker rings, and the ratio of roussel length to mantle length. The pattern of chromatophores on the head and the dentition pattern on the beak are similar for all three species and, therefore, of limited taxonomic value. The chromatophore pattern on the mantle, although potentially useful, is often obscured by damage to the mantle skin upon capture. The use of a combination of characters leads to positive identification of all sizes of these larvae. Identification of the larvae of these important cephalopods allows investigation into adult population parameters through larval sampling.

[1,2] This material is based on research supported in part by the National Science Foundation under Grant OCE-82-07754.

[1,3] Department of Oceanography.

Location of Thyrotropic Activity in the Pituitaries of Two Teleost Fish

LISA HELMS[14]

In teleost fishes, as in all vertebrates, thyrotropin (TSH), a glycoprotein hormone from the pituitary gland, is a principal factor regulating thyroid function. Nevertheless, teleosts are unique in having cells of the anterior pituitary, including those that produce TSH, segregated into distinct regions of the gland. A principal goal of the present study was to characterize the location of thyrotropic activity in the pituitaries of the tilapia (*Oreochromis mossambicus*) and the Hawaiian parrotfish (*Scarus dubius*). The presence of thyrotropic activity was assessed by using radioimmunoassay to quantify the release of thyroxine into medium following the *in vitro* coculture of the rostral and proximal pars distalis (RPD and PPD, respectively) and the pars intermedia (PI) with pieces of parrotfish thyroid. Within the parrotfish pituitary, the principal concentration of thyrotropic activity was found to occur in the PPD, although some activity was found in the RPD. No thyrotropic activity was identified within the PI. This pattern is in general agreement with descriptions of the distribution of thyrotropin cells based upon morphological criteria. In contrast, thyrotropic activity was absent from the RPD of the tilapia but was high in both the PPD and PI. Other investigators have described cells which show a positive periodic acid Schiff reaction (indicative of a glycoprotein hormone) in the PI of the tilapia. Together with these observations, our findings suggest that the PI may be an important location for thyrotropin cells in the tilapia.

Daytime Changes in Ovarian Factors of the Saddleback Wrasse, *Thalassoma duperrey*

KAREN HOFFMAN[15]

Evidence suggests that reproductive events in many fish species may be timed to coincide with specific phases of the tidal cycle. Variations in two ovarian factors, the gonadosomatic index (GSI) and the presence of mature, hydrated ova, were examined in order to characterize a potential association between the temporal progression of ovarian changes and the daytime tidal cycle in the Hawaiian endemic saddleback wrasse, *Thalassoma duperrey*. Female *T. duperrey* were collected by hook and line along the windward edge of a large patch reef in Kaneohe Bay, Hawaii, between sunrise and sunset prior to the full moon in October 1984, November 1984, and January 1985. Using the tidal cycle as a guide, sampling was split between two days. Replicate fish were sampled hourly. Changes in ovarian weights were calculated as the percentage of wet body weight devoted to gonad (gonadosomatic index). The presence of hydrated ova was determined by quick examination of ovarian tissue before fixation in Bouin's fixative. Mean GSI values of within-hour observations were calculated for each hour sampled around the high tide.

Preliminary results show gonadosomatic indices varying in a periodic pattern between sunrise and sunset in each of the three months sampled. Peak GSI values coincide with the

[14] Department of Zoology.
[15] Department of Zoology.

daytime high tide in October and November 1984 and precede the high tide in January 1985. The rise in GSI appears to be associated with the presence of hydrated eggs. Mature, hydrated eggs were observed from 1 hr before to 2 hr after high tide in October, whereas they were observed as early as 3 hr prior to high tide in November and January. However, no evidence of hydration appeared later than 2 hr preceding the high tide in January. A shift in the time of appearance of mature ova in January seems to coincide with the earlier GSI peak observed in January. Both GSI and hydration observations from this preliminary study suggest a readiness to spawn near the high tide in this coral reef species. The correlation observed between these factors and the daytime tidal cycle points to the potential importance of tidal forces to the reproductive success of the saddleback wrasse.

Effects of Silver Carp on Water Quality and Prawn Production in Prawn Ponds[16]

RICHARD S. J. WEISBURD,[17] E. A. LAWS, D. D. FOX, AND R. H. YORK

At present the commercial culture of giant freshwater prawns (*Macrobrachium rosenbergii*) in Hawaii is economically marginal at best. High costs of land, labor, and feed make increases in prawn production and improved efficiency of utilization of inputs essential to the survival of the Hawaiian prawn industry. Filter-feeding fish, silver carp (*Hypophthalmichthys molitrix*), have been stocked at low densities into 0.4-ha prawn growout ponds at Amorient Aquafarm (Kahuku, Oahu) to determine whether these planktivores improve pond water quality and prawn production.

Young silver carp were stocked at 20, 60, and 240 fish into each 0.4-ha pond in the experiment. A group of control ponds which received no fish was also monitored. Early-morning dissolved oxygen (DO) measurements were similar for all the treatments. DO increases from the early morning to late afternoon were larger in the ponds containing fish than in controls. The largest DO increases were in the 60-fish ponds. Primary productivity rates were measured in light and dark bottle incubations in one pond from each treatment weekly or semiweekly. From incubation data we have calculated rates of change of dissolved inorganic carbon and DO, as well as rate of incorporation of $H^{14}CO_3^-$ into particulates. Fish ponds exhibited greater primary productivity rates than did controls; the 20-fish and 60-fish ponds showed the highest rates. Particulate chlorophyll *a* was measured fluorometrically in all ponds either weekly or semiweekly. Treatment average chlorophyll *a* concentrations in the fish ponds exceeded those in the control ponds by 17.8%, 43.8%, and 35.4% respectively for the 20-, 60-, and 240-fish treatments. Treatment average prawn production from June through November in the experimental ponds increased with increasing fish density in a log-log relationship. Pond dissolved inorganic nutrient concentrations were measured by autoanalyzer periodically. Soluble reactive phosphate was scarce (<0.7 μm) in all ponds examined. Dissolved inorganic nitrogen averaged between 4.0 and 5.0 μm in control ponds and 20-fish ponds and between 1.0 and 1.5 μm in 60- and 240-fish ponds.

Our results show that stocking low densities of silver carp into commercial prawn ponds may increase both primary production and prawn production rates.

[16] Hawaii's Aquaculture Development Program and the Seagrant College Program are gratefully acknowledged for their support.

[17] Department of Oceanography.

Cytochemical Evidence for the Involvement of Cortical Alveoli During Preovulatory Hydration of Oocytes in a Teleost Fish

CHRISTOPHER D. KELLEY[18]

Preovulatory hydration of oocytes is a common phenomenon in teleost fishes that spawn pelagic eggs. Hydration is believed to impact neutral buoyancy and transparency to avoid predation. Cytochemical changes in oocytes undergoing hydration were examined in order to gain a better understanding of the physiological mechanisms underlying this process.

Ten female rock beauty angelfish (*Holacanthus tricolor*) were speared on St. Croix, U.S. Virgin Islands. The fish were obtained during morning, midday, and afternoon dives. The ovaries were removed, fixed in 10% buffered formalin, embedded in paraplast, and sectioned at 7 μm. Sections were stained with hematoxylin and eosin, Periodic Acid-Schiff (PAS) reagent, and Alcian Blue (AB) and examined by light microscopy.

Holacanthus tricolor spawns at sunset and, therefore, the three collections obtained oocytes in different stages of hydration. The histological characteristics of these oocytes suggest that during hydration, water initially appears between the chorion (zona radiata) and oocyte membrane forming a perivitelline space. This space disappears when the perivitelline water passes through the oocyte membrane and cortical cytoplasm to surround and diffuse among the yolk globules, which are simultaneously undergoing fusion. Subsequently, the water enters the partially fused yolk globules causing both their enlargement and the diffusion of their contents. This sequence of water movement through the membrane-bound "compartments" of the oocyte was repeated at least one more time prior to ovulation.

In some of the oocytes containing a perivitelline space, PAS- and AB- positive material was found in the space. The material, which stains as a glycoprotein, was in the form of granules or fused masses of granules adjacent to the oocyte membrane which diffused into a filamentous "meshwork" as it approached the chorion. The granules were identical in size and staining characteristics to those enclosed in the cortical alveoli located just inside the oocyte membrane. The distribution of the granular and filamentous forms of the glycoprotein suggests it was released by the cortical alveoli in a wavelike manner. Its appearance further suggests that, similar to what occurs at fertilization, the glycoprotein attracts water, forming a colloid that causes the expansion of the chorion away from the oocyte membrane, producing a perivitelline space. Its absence from the perivitelline space of many oocytes suggests that it rapidly becomes incorporated into the chorion or passes out through it.

The results of this study implicate cortical alveoli in the initiation of preovulatory hydration of oocytes in *H. tricolor*. Additional studies of other species are under way to determine whether preovulatory activation of cortical alveoli is a general mechanism in the hydration of teleost oocytes.

[18] Department of Zoology.

Social Organization of the Eastern Pacific Spotted Sharpnose Puffer, *Canthigaster punctatissima* (Tetraodontidae)

DONALD R. KOBAYASHI[19]

Sharpnose puffers (Tetraodontidae: Canthigasterinae) are a widespread group of reef fishes in tropical and subtropical seas. Although these fishes are seldom very abundant, their systematics are well understood and preliminary investigations into their ecology and behavior have suggested various levels of intraspecific sociality. This study examined the social organization and diet of a population of spotted sharpnose puffers, *Canthigaster punctatissima*, from the central Gulf of California.

The study was conducted in the summer of 1983 at a shallow (1–4 m) reef site near Punta Santa Inez, Baja California Sur. All puffers within the 150-m^2 site (approximately 20) were identifiable by tags (plastic beads) or natural markings. Visual surveys were made while snorkeling through the study site, and all intraspecific interactions (location, nature, and the identities of puffers involved) were documented and specific territories were mapped. Translocation and mirror experiments, as well as a removal experiment, were performed, after which 11 puffers were sampled for a laboratory evaluation of diet and gender.

Territories were maintained by both sexes in this sexually dimorphic benthic omnivore. These territories were aggressively defended against intruding puffers of the same gender. Large male puffers had territories which encompassed the territories of one to four small females. Male puffers routinely paired with all females in their territory at different times. Female puffers were only seen to pair with the male in whose territory they were located. Removal of a male puffer resulted in the overnight takeover of his territory and females by a neighboring male.

This population of *Canthigaster punctatissima* was apparently polygynous. The particular system appears to be best described as a "female-monopolization polygyny" in which males partition females, which are themselves spaced rather evenly over the reef. Some previous and ongoing studies of other tetraodontiform fishes have suggested gonochoristic (non-sex-changing) polygyny. There is some evidence that higher rates of predation on male puffers may lead to the observed skewed sex ratio.

Dietary Habits of Two Hawaiian Slipper Lobsters

COLIN J. LAU[20]

An investigation into the natural diets of two species of Hawaiian slipper lobsters, *Parribacus antarcticus* [Lund], and *Scyllarides squammosus* [H. Milne Edwards], by stomach content analysis (SCA) was conducted. Foregut evacuation rates were calculated for *Parribacus antarcticus* (Lund) to determine the amount of time required to pass a particular diet item from the stomach after consumption. Results indicate a bias for recognition of prey containing hard parts rather than soft-bodied prey. A modified

[19] Department of Oceanography.
[20] Department of Zoology.

index of relative importance (IRI) was calculated incorporating energetic values of prey consumed. IRIs were recorded as three-dimensional plots including data on prey type, percentage of total prey numbers, frequency of occurrence, percentage of prey biomass, and caloric content of prey items.

The two species of lobsters showed marked differences in types of prey consumed. *Parribacus antarcticus* can be characterized as an opportunistic feeder on benthic cryptofauna. Prey included actinarians, gastropods, chitons, polychaetes, stomatopods, holothurians, and asteroids. Low frequencies of occurrence were evident for most prey items. *Scyllarides squammosus* (H. Milne Edwards) exhibited a much greater degree of prey selection. Bivalves (*Isognomon* sp. and *Ostrea* sp.) comprised over 65% of the total diet. Prosobranch gastropods accounted for about 30% of the remainder. Within both species, there were little or no differences in prey consumed by slipper lobsters in the size ranges examined.

Protective Action of Calcium Entry Blockers in Endotoxin Shock

HING-CHUNG LEE[21]

Channels in cellular membranes which permit the influx of calcium from extracellular into intracellular fluid upon cellular excitation are blocked by a relatively new class of drugs known as calcium entry blockers (CEBs). The drugs have been reported to protect against myocardial necrosis caused by experimental myocardial ischemia, and the beneficial effect has been related to prevention of ischemia-induced calcium overload by the CEBs. Since circulatory shock can be expected to produce generalized tissue hypoxia, the possibility that CEBs might be beneficial in shock produced by endotoxin was investigated. In control male Wistar rats, a 10 mg/kg dose of *E. coli* endotoxin (Difco 0127:B8) produced bradycardia and a fall in blood pressure. Mortality 48 hr after endotoxin (10 mg/kg) was 84%. Three calcium entry blockers, verapamil, nitrendipine, and FR34235, administered intravenously 15 min before endotoxin, produced a dose-dependent reduction in mortality. The CEBs were less effective when given as post-treatment (15 to 30 min after endotoxin). Measurements of total tissue and mitochondrial levels in control rats revealed that endotoxin did not produce an increase in the calcium content of heart, lung, pancreas, small intestine, kidney, and aorta. Since cellular calcium levels did not increase in response to endotoxin, the protection induced by CEBs in endotoxin shock does not appear to be related to prevention of calcium overload. However, these experiments do not rule out the possibility of preventing calcium overload in discrete cells or tissues. In other experiments, the intravenous injection of endotoxin in control rats was found to produce changes indicative of disseminated intravascular coagulation. These effects included increased serum fibrin(ogen) degradation products (FDP), decreased plasma fibrinogen and blood platelet count, and microscopic findings of fibrin microthrombi in many organs. Pretreatment with CEBs antagonized the endotoxin-induced elevation in serum FDP and decrease in plasma fibrinogen. These observations suggest that the protective action of calcium entry blockers may be related to inhibition of disseminated intravascular coagulation caused by endotoxin.

[21] Department of Pharmacology.

Behavioral Factors Affecting the Growth Rate of *Macrobrachium rosenbergii*[22]

DANETT D. LI[23]

Social factors in freely interacting populations of *Macrobrachium rosenbergii* are believed to influence the heterogeneous individual growth rate (HIG) of these freshwater prawns. This preliminary study was undertaken to determine whether disproportionate feed acquisition occurs in *M. rosenbergii*, and if so, whether it is correlated with differential growth rates.

Four aquaria, each containing ten juvenile prawns, were used. Two of these aquaria contained prawns of equal lengths for observation of the development of HIG. The other two aquaria contained a variety of size classes for information on the behavioral interactions occurring when growth had already depensated. The following parameters were quantified, and regression analysis was done to determine significance: percentage of body weight gain, number of pellets eaten, percentage of body weight eaten, aggression, and the hoarding of feed.

The results show that there are significant relationships between many of these variables, indicating that disproportionate feed acquisition does occur in *M. rosenbergii* and influences growth rate. The primary factor influencing growth rate is thought to be aggression, as the more aggressive individuals hoard more feed. This behavior may enable them to eat a larger amount and, therefore, gain more weight.

The Influx of the Amazon River into the Caribbean Sea[24]

HILARY LYNN MAYBAUM[25]

The Amazon River contributes 10 to 18% of the world's total river runoff (Garrels, R. M., and F. T. Mackenzie 1971. *Evolution of Sedimentary Rocks*. New York: Norton.) and is, therefore, an important supplier of materials to the ocean. This research preliminarily defines the flow of estuarine waters of the Amazon into the Caribbean Sea by using a unique plankton classification method as the primary water mass tracer. Thirteen stations sampled along the leeward side of the Lesser Antilles and northern coast of South America from December 1980 to January 1981 were tested for Amazonian influence by using surface salinity, nutrient concentrations, and planktonic parameters as water mass tracers. Species of plankton specific to Amazon waters were detected. Analyses suggest two major pathways of influx occurring primarily with the Guiana Current between the Lesser Antilles and northern coast of South America and secondarily through Windward Island channels, especially between Martinique and St. Lucia. The principal factor governing abundance and east-to-west distribution of Amazon effluent appears to be seasonality of river discharge. Other influencing factors include upwelling, vertical mixing, and countercurrent flow. To derive a comprehensive model of Amazon inflow, future studies should include sampling during the predicted July–August rainy season, the period of maximum Amazonian discharge.

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[23] Department of Animal Science.

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[25] Department of Oceanography.

An Intertidal Algal Community Mosaic: Pattern and Permutation

KARLA J. MCDERMID[26]

Nine or more perennial seaweed species maintain mosaic patterns of distinct yet contiguous patches on low intertidal limestone solution benches in Hawaii. Permanent quadrats (0.25 m^2) were mapped each month for 15 months: algal patches were identified and numbered, and the amount of substratum covered by each patch was traced onto transparent Plexiglas plates centered over the quadrats. Areas delineated on the Plexiglas plates were computed with a digitizer to assess proportional cover.

Overall species diversity was relatively constant throughout the study period, though the patch pattern changed each month. Several species maintained between 10 and 50% cover each month: *Hypnea musciformis*, *Laurencia dotyi*, *Laurencia nidifica*, and *Sargassum obtusifolium*. *Acanthophora spicifera* and *Gelidiella acerosa* were present each month but occupied less area. Infrequently occurring taxa, such as *Hypnea chordacea*, *Laurencia majuscula*, and green algae including *Ulva* sp., were considered rare. Although certain taxa were consistently dominant each month, the individual patches of these taxa displayed varying degrees of transience—some were established, grew, and disappeared within 1 month whereas others survived for over 7 months. Daily patch growth rate and patch persistence varied among the species and their patch size classes. Statistical analysis revealed that for *Laurencia dotyi* and *L. nidifica* larger patches had a significantly greater percentage of patch survival from one month to the next than did patches of smaller size classes.

Reproductive state of each species was assessed by random sampling outside the quadrats. Only asexual plants (tetrasporophytes) and vegetative growth were shown by the majority of the red algal species, which constituted five of the six dominant taxa.

The data analyzed thus far indicate that intertidal algal community structure in subtropical Hawaii shows little response to seasonal changes such as water temperature, air exposure, or light, and when compared to similar communities in southern California it shows fewer species yet the same lack of seasonal trends in species composition and abundance. Individual algal patch dynamics may reflect the effects of inter- and intraspecific interactions in a natural community where space is a limiting resource.

Photic Responses of the Larvae of *Phestilla sibogae* (Opisthobranchia: Nudibranchia)[27]

STEPHEN E. MILLER[28]

Recent work on larval behavior has demonstrated greater complexity than indicated by earlier reports. Temporal changes in phototaxis of lecithotrophic larvae of *Phestilla sibogae* are discussed here. In separate horizontal and vertical tests, larval distributions were recorded in response to changes in light intensity (43, 130–170, 890–900 $\mu E/m^2/sec$) and larval age. Additional observations of individual larval behavior in response to horizontally directed light (900 $\mu E/m^2/sec$) allowed quantification of path direction, stops, and spirals during swimming. Horizontal tests showed young larvae (days 5

[26] Department of Botany.

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[28] Department of Zoology.

to 7 postfertilization) to be highly photopositive. Positive phototaxis decreased with age, showing the greatest change in mid-aged larvae (days 8 and 9). In vertical tests young larvae were initially photopositive on exposure to light, but positive phototaxis decreased with time (< 60 min). Mid-aged larvae showed a similar but less pronounced positive phototaxis. Higher light intensity increased positive phototaxis by mid- and old-aged larvae (days 10 to 11) in both horizontal and vertical tests. Observations of individual larvae showed that the frequency of stops and spirals increased with age, time spent swimming decreased, and swimming speed remained constant.

Photic responses of many marine invertebrate larvae have been characterized as photopositive at hatching followed by negative phototaxis or photo-indifference during later larval development. Ecological counterparts to these responses are growth and dispersal of young larvae incapable of metamorphosis followed by substrate selection, settlement, and metamorphosis of older larvae. The data presented here suggest that simple on/off photic response may not be the general case and would inadequately address the ecological requirements for dispersal and successful settlement. A photic response that varies with age, light intensity, and recent photic experience may be of greater adaptive value.

Genetic Variation in Human Aldehyde Dehydrogenase

STUART NEWFELD[29]

NAD-dependent aldehyde dehydrogenase (ALDH, E.C. 1.2.1.3) is one of the principal enzymes responsible for human alcohol metabolism. Investigators have demonstrated apparent genetic variation both within and between ethnic groups for two major isozymes of ALDH (identified by their isoelectric points at pH 4.9 and pH 5.3). This variation is detected through lack of pH 4.9 isozyme activity in samples from a percentage of Japanese and Chinese donors. These observed differences have been linked to differences in alcohol consumption, to the flushing response, and to protection against alcoholism. However, because the most common method of obtaining active ALDH for assay employs autopsy or biopsy tissue, these surveys of phenotypic variation have been limited.

This presentation reports a new method for demonstrating ALDH isozymes in normal human serum or plasma. Studies indicate that the two major isozymes can be identified and the common Oriental variant detected. To confirm these results, ALDH from an autopsy liver was isolated and purified. The liver ALDH isozymes are identical in isoelectric point to those found in human blood. In addition, subsets of isozymes clustered around the pH 4.9 and pH 5.3 isoelectric points have been demonstrated which may represent previously unreported allozymes of the two isozymic forms.

The method of detecting isozyme variation in human serum or plasma and the protocol for isolating purified ALDH isozymes from autopsy liver are modifications of the technique reported by Ikawa et al. (J. Biol. Chem. 258:6282–6287, 1983). Substituting "spin" columns for large chromatography columns allows classification of ALDH isozyme profiles approximately 24 hr after venipuncture. To date, 38 samples of plasma from Japanese donors were surveyed for presence or absence of pH 4.9 isozyme activity. By this method, approximately 40% of the samples contained no detectable pH 4.9 activity. This finding agrees with previous estimated frequencies of this variant. Employing this technique to assess the frequency of the variant in unsurveyed populations, rapid and reliable results can be expected.

[29] Department of Genetics.

Photosynthetic Light Acclimation to Light Quality and Quantity in *Leucaena leucocephala*

MATTHEW H. PERRY[30]

Photosynthetic acclimation to changes in quantity and quality of light in "Hawaiian Giant" koa haole (*Leucaena leucocephala*) was studied to determine the extent and mechanism of acclimation in koa haole. The Hawaiian Giant cultivar is of interest because of its high yield and arboreal habit.

Light quantity was varied by growing plants in a greenhouse under varying thicknesses of neutral plastic shade cloth, which resulted in integrated daily photosynthetic photon flux densities (PPFD) of 27, 17, 8.1, 4.0, and 1.6 mol $m^{-2}d^{-1}$. Light quality was varied by growing plants under a green filter, which resulted in an integrated PPFD of 3.7 mol $m^{-2}d^{-1}$, and neutral shade cloth which resulted in a nearly equal level of integrated PPFD. The shade under the green filter is similar to the shade under a green leaf (plant canopy) because the filter absorbs red light but not far-red light. Gas exchange rates were measured using a LICOR LI-6000 infrared gas analyzer. Chlorophyll content was measured according to Arnon. Stomatal density was measured using cleared leaflets which were stained with safranin.

When plants were grown under neutral shade cloth, they responded in a manner typical of plants which respond to light quantity. That is, the light-saturated net photosynthetic rate ($P_{n\,max}$), dark respiration, light compensation point, stomatal density, stomatal conductance, and leaf thickness were lower, and chlorophyll content was higher, when plants were grown in low PPFD.

A comparison of plants grown under green shade and neutral shade at equal integrated PPFDs showed that koa haole responds to green shade differently than neutral shade. Neutral-shade-grown plants had higher $P_{n\,max}$, leaf area, and leaf thickness. The relative importance of light quantity and quality in causing acclimation is not clear since it was not possible to vary them independently. It is concluded that koa haole is capable of extensive acclimative responses, because it responds to changes in light quantity and to simultaneous changes in light quantity and quality.

The Effects of Cadmium on Growth and Development of Larval Bivalves

AMY HUFFMAN RINGWOOD[31]

Bivalves have been used extensively to study the effects of elevated metal concentrations in marine ecosystems. Although much is known about the responses of adult bivalves, the effects on embryos and larvae have received little attention. Thus, experiments were designed to study the effects of cadmium on the embryos and larvae of the Hawaiian bivalve *Isognomon californicum*. LD_{50} (the concentration required to cause 50% mortality) was used as a comparative tool to determine the relative sensitivities of the different developmental stages. Embryos were found to be more tolerant than larvae. Using a range of cadmium concentrations from 1.0 μg/L (ppb) to 10.0 mg/L (ppm), early cleavage patterns were not disrupted at even the highest concentrations. At both 5 and 10

[30] Department of Botany.
[31] Department of Zoology.

ppm, however, development was arrested at the trochophore stage as the embryos failed to develop into shelled veligers. Cadmium may affect morphogenesis rather than cell division.

Concentrations as high as those required to cause death in 48 or 96 hr are rarely encountered in nature. Laboratory-reared larvae were exposed to sublethal concentrations of cadmium, added as $CdCl_2$ to filtered seawater. The effects on growth, as determined by changes in size and weight, were recorded. Growth was reduced at concentrations as low as 20 ppb. The effects of sublethal concentrations are certainly more meaningful ecologically. Reduced larval growth would either require a longer period in the plankton or result in decreased size at settlement, either of which may reduce long-term survivorship.

The Population Structure of a Tropical Mytilid in Two Different Intertidal Environments

THOMAS L. SMALLEY[32]

The small, endemic Hawaiian mussel *Brachidontes crebristriatus* is common to abundant on intertidal limestone and basalt shorelines of the windward Hawaiian Islands. This mytilid has also been reported to form dense subtidal beds to depths of 10 m. Although *B. crebristriatus* may be abundant, it exhibits a patchy distribution in space both within and between intertidal habitats. This paper describes some aspects of the population structure and dynamics of *B. crebristriatus* from two different intertidal habitats. The two study sites, Diamond Head Beach Park and Kahana Bay, Oahu, are characteristic of two major habitat types in which *B. crebristriatus* is abundant. These habitats differ in their physical and structural features.

Population densities of *B. crebristriatus* differ significantly between these two habitat types, reaching densities up to 32,000 and 57,000/m^2 at Diamond Head Beach Park and Kahana Bay, respectively. In addition, the physical structure (three-dimensional growth form) of these populations varies between habitat types. At Diamond Head Beach Park, *B. crebristriatus* cover hundreds of square meters of a horizontal, midintertidal limestone platform. Most individuals occur in a single layer, embedded in a short algal turf and attached firmly to the limestone substratum. At Kahana Bay, suitable substrata are less abundant and the total surface area covered by *B. crebristriatus* is less than 50 m^2. At this site the mussels are restricted to a 1-m fringe of a gently sloping concrete boat ramp (low and midintertidal) and to the upper surfaces (horizontal and vertical; midintertidal) of relatively small basalt boulders sitting on a sand/silt substratum. In contrast to the Diamond Head population, *B. crebristriatus* at Kahana Bay form clumps several individuals deep. Mean shell length and flesh dry weight also differ between populations—those from Kahana Bay are significantly larger and heavier. Size frequency distributions vary from month to month at both sites and reflect periods of recruitment. Sex ratios at both sites fluctuate around a 1 : 1 value and do not vary significantly over time within or between sites. The presence of ripe animals and newly settled individuals at both sites, from March 1984 to February 1985, suggests year-round reproductive activity and recruitment in these populations. Judging from gonadal condition and the appearance of large numbers of juveniles

[32] Department of Zoology.

in December 1984 and January–February 1985 there appears to be at least one major peak in reproduction and recruitment. Between-habitat differences in reproductive activity also occur—the Kahana Bay population exhibits a greater reproductive potential and greater recruitment.

It is suggested that these differences can be explained by between-site variability in environmental features such as food availability, wave impact, and structural features of the habitat. The relative importance of these factors in structuring populations of *B. crebristriatus* has yet to be experimentally determined. (Michael G. Hadfield has kindly provided laboratory space for which I am most grateful.)

Intracellular Changes in Parrotfish Thyroid After *in Vitro* Bovine Thyrotropin Stimulation

CAROL JOHNSON SMITH[33]

Six groups of thyroid glands of *Scarus dubius* were examined and compared by electron microscopy after a 4-hr culture with graded doses of bovine thyrotropin (bTSH). Five doses of bTSH were used encompassing the full range of the dose-response curve developed for this tissue. Upon electron microscopic examination, micrographs were taken randomly and at the same magnification, and three intracellular inclusions were quantified. The relative surface density of rough endoplasmic reticulum and the relative surface area of lysosomes and engulfed colloid droplets were recorded for each group. Three treatment groups—(1) control, no bTSH; (2) tissues exposed to 1 mIU/mL bTSH; and (3) tissues exposed to 2 mIU/mL bTSH—did not differ from each other in the quantified organelles or in general appearance. Overall, these three groups were similar in appearance to the ultrastructure described in other teleosts except for a lack of flagellated cells. Compared to the first three groups, treatment with 5 mIU/mL bTSH (group 4) increased the density of rough endoplasmic reticulum and the proportion of cell area occupied by lysosomes and engulfed colloid. Group 4 also possessed either more microvilli or pseudopods at the lumenal surface of the follicular epithelium. In group 5, exposure to 10 mIU/mL bTSH showed an even greater increase in surface density of rough endoplasmic reticulum and in surface area occupied by lysosomes and engulfed colloid droplets. The apical portion of this group was highly irregular, commonly displaying pseudopods. Group 6 (20 mIU/mL bTSH) showed a decline in cytoplasm in comparison to group 5 with many epithelial cells breaking apart. A few cells in this group were still intact but contained huge engulfed colloid droplets which extended from the basal to apical borders. In comparison to the dose-response curve developed for the tissue, thyroid hormone (T4) first increases when the tissue is cultured with a dose of 2 mIU/mL bTSH (for 4 hr). Yet ultrastructural changes do not occur at this dose, so there appears to be a lag between release of thyroid hormone and augmentation of organelles. The peak of T4 released is achieved when the tissue is cultured with a dose of 10 mIU/mL bTSH. The surface area and density of the measured organelles also peak at this dose. The dose-response curve shows a decline in T4 release when the tissue is cultured with 20 mIU/mL bTSH. The decline may be due at least in part to the cell lysis seen in this group. Since the few intact cells contained huge engulfed colloid droplets, the lysis probably occurred from the engulfment of more colloid than the membranes could contain.

[33] Department of Zoology.

Distribution of the Symbiotic, Colonial Ascidian *Diplosoma similis*: Limitations Imposed by Light Intensity

DOUGLAS S. STONER[34]

Laboratory experiments were carried out to test the hypothesis that light limits the distribution of the symbiotic, colonial ascidian *Diplosoma similis* and to examine the mechanisms by which *Diplosoma* photoadapts to its environment. Light- and shade-acclimated colonies were grown for 1 month under three light treatments: 5, 20, and 100% ambient sunlight intensity. To control for water flow, water temperature, and food regime, all colonies were grown in a single tank and received water from the same source.

Growth and survivorship were measured to determine the extent to which light limits *Diplosoma*'s distribution. Growth was found to be highest in the intermediate light treatment and lowest in the low light treatment. Survivorship was lowest in the high light treatment, and within this treatment the shade-acclimated colonies had significantly lower survivorship than the light-acclimated colonies. Regardless of initial acclimation environment, survivorship of *Diplosoma* was facilitated by movement of colonies away from full sunlight.

In response to increased light intensity, the zooid density, concentration of UV-absorbing compounds, and production of visible pigment granules all increased. The chlorophyll *a*/*b* ratio of the algal symbiont *Prochloron* sp. also increased.

These results suggest that although *Diplosoma* possesses a number of adaptations which allow it to live in habitats exposed to light, its distribution is still limited by light to habitats which are neither highly shaded nor receive direct sunlight.

Food and Competitors as Determinants of Feeding-Territory Size in Coral-Feeding Butterflyfishes: An Experimental Analysis[35]

TIMOTHY C. TRICAS[36]

Models that predict the effects of food and competitor abundances on feeding-territory size were tested by experimental field manipulations on the butterflyfish, *Chaetodon multicinctus*. This species is a monogamous obligate corallivore that spends more than 90% of its time in feeding activities and less than 5% vigorously defending well-defined contiguous territories against intrusion by other conspecifics.

The "sufficient food" hypothesis, which predicts that territory size is adjusted as an inverse function of food abundance, was tested by manipulations of coral food supplies. When coral abundance was reduced, residents expanded their territories by encroachment into neighboring areas. Increased food supply, however, did not result in a complementary decrease in territory size. Instead, fish attempted to defend added food resources within or slightly beyond the original boundaries and were limited by aggressive interactions with other conspecifics attracted to supplemental food resources. Thus, the major prediction of the "sufficient food" hypothesis was not supported by the experiments.

[34] Department of Zoology.

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[36] Department of Zoology.

The "competitor constraint" hypothesis, which predicts that territory size is adjusted as an inverse function of competitor abundance rather than food supply, was tested by manipulations of conspecific fish. When adjacent neighbors were removed, foraging areas of experimental pairs increased. When increased intrusion rates into experimental territories were simulated by the addition of bottled conspecifics within borders, defended areas decreased. Furthermore, the critical assumption of this hypothesis, that competitor abundance varies directly with food supply, is met by this system as shown by the attraction of large numbers of conspecifics to supplemental corals. Thus, the "competitor constraint" hypothesis was not rejected.

It is concluded that when intraspecific conflicts over food resources occur, conspecific competitors are a more significant determinant of the spatial character of territories than is the abundance of food resources. These results also support predictions of more comprehensive models for energy maximizers that address the interaction of food and competitors as determinants of optimal feeding-territory size and activity budgets.

Comparative Anatomy of Some Parents and Hybrids in the Hawaiian Silversword Complex

INSUN YUN (KIM)[37]

Comparative anatomy of the leaves of five species of the Hawaiian silversword complex (*Argyroxiphium grayanum*, *Dubautia scabra*, *D. knudsenii*, *D. ciliolata*, and *Wilkesia gymnoxiphium*) and four intergeneric and interspecific hybrids (*A. grayanum* × *D. scabra*, *D. knudsenii* × *D. scabra*, *D. ciliolata* × *D. scabra*, *W. gymnoxiphium* × *D. scabra*) has been studied in order to explore the variation pattern and evaluate the relationships among them. Foliar morphology, leaf tissue organization, and various characters of stomata and trichomes were compared using cross and paradermal sections and clearings of leaves.

In most cases comparisons of parents and hybrids revealed statistically significant differences in leaf size, leaf tissue organization, occurrence and distribution of adaxial and abaxial stomata, and marginal and surface trichomes. It appears that hybrids demonstrate intermediate status in their foliar characters, indicating that these characters have been inherited from both parents. However, the numerical values were sufficiently different to allow their recognition in most cases.

[37] Department of Botany.